THE PROBLEM OF DIFFERENCES OF OFFSPRING IN RECIPROCAL CROSSES OF DROSOPHILA[1]

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In general, reciprocal crosses between genetically different groups of organisms yield identical hybrids. In some cases, however, the offspring from reciprocal crosses are different from one another in spite of like nuclear constitution. Such cases are of particular interest, since they bear on the problem of the role of extranuclear factors in development and heredity.

In *Drosophila melanogaster* a few examples have been described in which the developmental fate of a zygote depends in part on the genetic constitution of the mother. It seemed possible to obtain further data. A series of reciprocal crosses was performed and the expression of a sensitive indicator phenotype, caused by the mutant "Dichaete," was determined. Several of these crosses seemed to give evidence of a maternal effect. However, further inquiry showed that the significant differences between the outcome of reciprocal crosses were at least partly due to inhomogeneity within each cross, and that no conclusion as to the existence or non-existence of maternal effects was justified. The data and their analysis presented here serve to emphasize the need for detailed statistical study of the range of variability within, as well as between, sets of data under comparison.

Materials and Breeding Methods

The mutant phenotype "Dichaete" (*D*, 3-40.4-41 ±) is a very sensitive indicator of external and internal conditions prevalent during the development of *Drosophila melanogaster* (Sturtevant, 1918; Plunkett, 1926). A quantitative measure of such conditions may be obtained by counting the number of dorsocentral bristles on the thorax of Dichaete flies. Normal flies usually have four dorsocentrals while Dichaete flies may have from zero to four.

Plunkett has already given some data showing that the mean number of dorsocentrals is not significantly different in the Dichaete offspring from reciprocal crosses between Dichaete and normal flies. The procedure followed in the present study consisted in reciprocal crossing of Dichaete and non-Dichaete flies, the latter of which contained some other mutant genotype. In other words, the expression of Dichaete was measured in populations which not only differed in the maternal or paternal origin of the Dichaete genotype but also in the origin of the egg from a mother either free or in possession of some other mutant genotype. The mutants used in combination with Dichaete were vestigial (2-67.0) Minute (2) (2-12.9 ±),

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Minute (3) *w* (3-79.7), Minute 4 (4), and polychaetoid (3-39 ± 4). These mutants hereafter will be referred to as *M*-2, *M*-3, *M*-4, *pyd*, *vg* and *D* (Dichaete). The choice of mutants used was based on the following considerations: (1) *vg*. Hersh and Ward (1932) have reported that *vg*/+ heterozygotes possess slightly different wing dimensions, dependent on the direction of the parental cross, vestigial by normal. It was thought that the maternal effect indicated by these findings might express itself also in a modification of the bristle reaction in *D* flies. (2) Minutes. The effect of a Minute constitution consists not only in the morphological modification of bristle size but also in a striking prolongation of larval growth (Dunn and Mossige, 1937). The physiological mechanism underlying this developmental prolongation is unknown, but it seemed worthwhile to test whether egg cells derived from Minute females differed from egg cells derived from non-Minute females in their effect on the Dichaete reaction. (3) *pyd*. The use of this mutant was based on the supposition that its action toward increase of bristles may possibly result in a difference of egg cytoplasm from *pyd* as opposed to normal females.

The initial problem was to obtain seven stocks which were highly alike except for the mutants to be tested (*D*, *M*-2, *M*-3, *M*-4, *pyd*, and *vg*). The method was to replace whole chromosome "marked" with dominant mutant genes by homologous chromosomes of the isogenic wild type stock "Canton-S." A laboratory stock with dominant marked genes in the second and third autosome, of the genotype +/+ ; *Cy*/*Pm*; *Sb*-*C*/*H* * was crossed with each stock of *D*, *M*-2, *M*-3, *M*-4, *pyd*, and *vg* and their progeny outbred for three generations with Canton-S stock. In case of crosses involving *D* or the Minutes, when all marked chromosomes, as well as the *X* and *Y*, had been replaced by homologous Canton-S chromosomes, the mutant female of the resultant stock was chosen as one parent of each subsequent cross with Canton-S males. This was done in order to produce as much homogeneity as possible in the mutant chromosomes by allowing crossing-over to occur. The crosses described above were maintained for fourteen generations; subsequent flies were maintained by mass matings with all female parents of such mass matings selected from the same bottle. The Canton-S flies referred to were reproduced in each generation by pair matings from a single bottle.

A slightly different breeding method was used with *vg* and with *pyd*, because the former is a recessive and the latter a very weak dominant; it was necessary to inbreed flies from the fourth generation in order to obtain the *vg* or *pyd* phenotype (fully expressed in homozygous condition only). Thereafter, the *vg* and *pyd* flies were alternatingly outbred to Canton-S and inbred until the fourteenth generation was reached and the *vg*/*vg* and *pyd*/*pyd* flies were used in the experimental crosses.

The experimental flies were obtained from reciprocal crosses between the approximately isogenic stocks of Dichaete and of *vg*, *pyd*, *M*-2, *M*-3, and *M*-4. Reciprocal crosses were also made between Dichaete and non-Dichaete siblings. Altogether, twenty crosses involving 456 cultures were made.

All crosses were maintained in a seven-shelved incubator ventilated by a forced draft fan. The temperature was maintained between 24.5 and 25.0 degrees Centi-

* Symbols used here refer to mutant genes as follows: *C* = crossing-over suppressor, *Cy* = curly wings, *H* = lack of certain hairs, *Pm* = plum eye color, *Sb* = stubble bristles. For further details see Bridges and Brehme (1944).

grade throughout the whole experiment. Crosses were indiscriminately mixed on the shelves, thus insuring that no one group of bottles occupied a "favored" position.

Two culture methods were used. (1) In the "bottle" method, virgin females were collected over a two-day interval and each was put with a male in a 20 × 90 mm. shell vial containing a piece of agar-molasses substrate (unyeasted) to supply moisture. At the end of the two days, the pairs of flies were transferred to ordinary half-pint milk bottles with the usual cornmeal-molasses food (unyeasted). After an additional three days the parents were shaken out and the bottles returned to the incubator. (2) The "egg-count" method differs in a few respects from the "bottle" method. Virgin females were collected as before and pair-mated in 20 × 90 mm. shell vials for two days. The pairs were then removed and put into half-pint milk bottles containing egg-laying dishes. These were rectangular metal dishes, 60 × 30 mm. long and wide, and 10 mm. deep, filled with food. The same substrate was used as above. Egg counts were then made. When 120 eggs had been deposited on the surface of the substrate, 12–36 hours later, it was transferred to a 4½-inch finger bowl with yeasted food medium. The bowl was then covered with cotton gauze and another finger bowl and placed in the incubator. When all larvae had pupated they were transferred to bottles and remained there until eclosion.

Effect of Culture Method on Population Density

Considerable variability in size of population per bottle and in average population per cross was found with either culture method. The area of substrate per larva (as judged by flies surviving to eclosion) also differed greatly. Table I contains relevant data.

Table I

Effect of culture method on population density

	Egg-count method. Area of agar surface 9710 mm.²		Bottle method. Area of agar surface 2633 mm.²	
	Total population surviving	Area per surviving larva	Total population surviving	Area per surviving larva
Least densely populated bottle	33	294	75	35
Least densely populated cross (average)	69	141	100	26
Average of all crosses	82	118	115	23
Most densely populated cross (average)	95	102	130	20
Most densely populated culture	123	79	189	14

The two methods offered very different conditions of existence to the larvae. If these conditions had any significant effect upon bristle number, identical crosses cultured by the two methods would not be expected to yield comparable results.

Analytical Methods

The Dichaete offspring of the experimental crosses were classified by sex and number of dorsocentral bristles. No distinction was drawn between *M* and M^+ flies. As an index, the mean number of posterior dorsocentral bristles per hemithorax was computed separately for each sex in each bottle. In computing the index, one-half the number of one-bristle flies were added to the total number of two-, three-, and four-bristle flies, and this resulting sum divided by the number of flies of that sex in that bottle. This method of calculating posterior dorsocentral bristle frequency is justified by the data of Plunkett (1926) and Walker (1941). According to these workers, the effect of Dichaete is to remove the anterior dorsocentral bristles first and the posterior dorsocentral bristles secondarily, if the effect is strong enough. This means that if two bristles remain, they are practically always the posterior dorsocentrals, and if one bristle is present it is with about equal frequency the right or left posterior dorsocentral. As an overall measure of bristle frequency (in one sex) in a particular cross, a mean bottle index ($\overline{P}_{\text{cross}}$) was calculated in the same manner as for a single bottle. A summary of cross indices is shown in Table II.

Table II

Bristle frequencies (as measured by presence of posterior dorsocentrals per hemithorax; wild-type = 1) for Dichaete progeny of the experimental crosses. "A" and "B" refer to reciprocal crosses, the former denotes the cross in which the female *parent is Dichaete and the latter denotes the cross in which the* male *parent is Dichaete.*

Experimental cross number	Mutant used to test for "maternal" effect	Female progeny		Male progeny	
		A	B	A	B
		Mutant father	Mutant mother	Mutant father	Mutant mother
		Cross index		Cross index	
1*A* and 1*B*	*vg* (bottle method)	0.704	0.752	0.529	0.635
**2*A* and 2*B*	*vg* (egg-count method)	0.774	0.710	0.681	0.403
3*A* and 3*B*	*M*-4 (bottle method)	0.432	0.363	0.289	0.180
4*A* and 4*B*	*M*-3 (bottle method)	0.585	0.601	0.423	0.445
**5*A* and 5*B*	*M*-3 (egg-count method)	0.824	0.903	0.625	0.605
6*A* and 6*B*	*M*-2 (bottle method)	0.669	0.529	0.500	0.425
7*A* and 7*B*	*pyd* (bottle method)	0.697	0.596	0.660	0.445
8*A* and 8*B*,	Dichaete* (egg-count	0.855	0.853	0.783	0.718
9*A* and 9*B*,	method)	0.779	0.793	0.599	0.523
10*A* and 10*B*		0.843	0.891	0.671	0.709

* These experiments were carried out within the Dichaete stock itself; *D*/*C* females and males were crossed reciprocally with *C*/*C* males and females.

** These crosses done by the "bottle" method and repeated later by the "egg-count" method.

Whether the indices (for the same sex) of the reciprocal crosses were or were not significantly different was determined in three steps:

(1) for each sex ($\bar{P}_{\text{females}}$ and $\bar{P}_{\text{males}}$), a joint mean of the two reciprocal crosses was calculated from the combined data of the two crosses in the same manner as for a single bottle.

(2) the bottles above and below the joint means, sexes considered separately, were entered in a four-fold table.

(3) this table was tested for homogeneity by the usual χ^2 method. The relevant data for all crosses are summarized in Table III which shows the probability

TABLE III

χ^2, n, values for distribution of bottles whose indices fall above and below the joint mean ($\bar{P}_{\text{females}}$ and $\bar{P}_{\text{males}}$) for each reciprocal cross.

Crosses compared			Female progeny		Male progeny		Combined progeny	
No.	♀ × ♂		$(n=1)$ χ^2	P	$(n=1)$ χ^2	P	$(n=2)$ χ^2	P
1A	*D/C* ×	*vg/vg*	5.127	0.025	4.282	0.042	9.409	0.010
*1B	*vg/vg* ×	*D/C*						
*2A	*D/C* ×	*vg/vg*	5.432	0.020	24.966	0.003	30.398	<0.010
2B	*vg/vg* ×	*D/C*						
*3A	*D/C* ×	*M-4/C*	2.785	0.100	2.115	0.160	4.900	0.089
3B	*M-4/C* ×	*D/C*						
4A	*D/C* ×	*M-3/C*	2.131	0.154	1.054	0.306	3.185	0.204
*4B	*M-3/C* ×	*D/C*						
†5A	*D/C* ×	*M-3/C*	3.343	0.072	0.016	0.900	3.359	0.190
**5B	*M-3/C* ×	*D/C*						
*6A	*D/C* ×	*M-2/C*	9.185	0.007	6.417	0.011	15.602	<0.010
6B	*M-2/C* ×	*D/C*						
*7A	*D/C* ×	*pyd/pyd*	13.616	<0.010	18.169	<0.010	31.885	<0.010
7B	*pyd/pyd* ×	*D/C*						
*8A	*D/C* ×	*C/C*	0.744	0.407	3.702	0.056	4.446	0.115
8B	*C/C* ×	*D/C*						
†9A	*D/C* ×	*C/C*	0.628	0.444	1.563	0.219	2.191	0.392
**9B	*C/C* ×	*D/C*						
10A	*D/C* ×	*C/C*	3.478	0.067	0.965	0.332	4.443	0.117
*10B	*C/C* ×	*D/C*						

* Cross giving higher index for progeny of both sexes.
** Cross giving higher index for female progeny.
† Cross giving higher index for male progeny.

that such a distribution above and below the joint index could be a chance occurrence.

Of the mutants tested, three—*pyd, vg,* and *M-2*—have significantly different indices in reciprocal crosses; two, *M*-3 and *M*-4, do not, nor does Dichaete itself.

One totally unexpected feature of these results is that the difference between reciprocal crosses involving *vg* was of opposite sign in the two trials, but significant in each case. In crosses $1A(D/C♀ \times vg/vg♂)$ and $1B(vg/vg♀ \times D/C♂)$ both male and female offspring had higher indices where the vestigial parent was the *mother;* in crosses $2A(D/C♀ \times vg/vg♂)$ and $2B(vg/vg♀ \times D/C♂)$ each sex showed a larger index where the vestigial parent was the *father.* Among many possible explanations, three seem most worthy of consideration. (1) Inefficient statistical methods may have been used. It may be recalled here that the general purpose of statistical tests of the reality of observed differences is to predict whether similar differences would be expected in repetitions under comparable conditions. Predictions are based here on the variation of bottle indices *within* an experiment. In the present case the statistical test of either *one* experiment of a pair leads to the expectation that the other experiment should yield a difference of the same sign, if it has been performed similarly. The other, however, gives opposite results. (2) There may have been a difference in procedure between the two experiments of a sort which, in general, reverses the direction of difference between reciprocal crosses involving *vg* and Dichaete. In fact crosses $1A(D/C♀ \times vg/vg♂)$ and $1B(vg/vg♀ \times D/C♂)$ were raised by the bottle method and had average population densities of 4.7 and 4.4 larvae per cm.[2] (as judged by the number surviving to eclosion); crosses $2A(D/C♀ \times vg/vg♂)$ and $2B(vg/vg♀ \times D/C♂)$, raised by the egg-count method, had densities of 0.85 and 0.91 larvae per cm[2]. (3) There may have been a difference in material between the two experiments. In fact the vestigial parents used in the two trials were not of undoubtedly identical genotype. In maintaining the *vg* and Canton stocks, a contamination was noticed in both of them between the first and last *vg* experiments. The Canton stock was re-obtained from a laboratory stock which had come originally from the same stock as the first Canton stock but had been inbred by mass matings instead of pair matings. A stock of vestigial was obtained from the same source as before and was made approximately isogenic by the same procedure as before. On the whole, then, explanation (3), difference in stock, seems relatively improbable.

Explanation (1), inefficient statistical method, also may reasonably be excluded: the method is standard and its prediction about repetitions is upheld in the one cross which was repeated without change in culture method. Three sets of reciprocal crosses were made between Dichaete and non-Dichaete siblings. In each set of reciprocal crosses the indices for both sexes were found not to be significantly different. The prediction from the statistical tests is also fulfilled in one case where the culture method was changed, that is, crosses $4A(D/C♀ \times M\text{-}3/C♂)$ and $4B(M\text{-}3/C♀ \times D/C♂)$ were done by the bottle method and their indices (for both sexes) were found not to be significantly different. Later, this same pair of reciprocal crosses was repeated (cross $5A$: $D/C♀ \times M\text{-}3/C♂$ and cross $5B$: $M\text{-}3/C♀ \times D/C♂$) and the progeny raised by the egg-count method. Here, as before when the bottle method was used, neither sex showed indices significantly different from those of their reciprocal crosses.

Thus explanation (2), effect of culture method, seems to be the only one of the three leading possibilities which cannot be reasonably excluded. That it

should be *expected* to bring about a reversal of the difference between reciprocal crosses is not obvious from the literature on bristle phenotypes. That it may actually have done so can scarcely be decided without some study of the relation between bristle index and the factors which change with varying culture conditions. It is with this problem that the following section is concerned.

Factors Affecting the Bristle Index

Three factors affecting the bristle index will be considered.

(1) *Differences within crosses.* Whatever the factors may be which affect the bristle frequency in the present material, they do not operate identically even through a group of bottles prepared at the same time, containing progeny from parental pairs of the same genotype, and all incubated within 0.5 degrees Centigrade of the same temperature. This fact was established by making χ^2 tests for homogeneity for the female indices within each of the twenty experimental crosses. The results of these tests are listed in Table IV. Altogether, in twelve

Table IV

χ^2 treatment of the comparison of bottle and group indices of the female progeny within crosses 1A–10B

Cross No.	♀ × ♂	χ^2	n	P
1A	D/C × vg/vg	94.179	34	<0.010
1B	vg/vg × D/C	64.463	16	<0.010
2A	D/C × vg/vg	55.793	29	<0.010
2B	vg/vg × D/C	105.025	35	<0.010
3A	D/C × M-4/C	47.372	30	0.024
3B	M-4/C × D/C	12.815	17	0.747
4A	D/C × M-3/C	104.200	37	<0.010
4B	M-3/C × D/C	108.071	40	<0.010
5A	D/C × M-3/C	18.895	18	0.407
5B	M-3/C × D/C	9.082	14	0.823
6A	D/C × M-2/C	132.111	29	<0.010
6B	M-2/C × D/C	143.243	45	<0.010
7A	D/C × pyd/pyd	72.103	31	<0.010
7B	pyd/pyd × D/C	93.845	45	<0.010
8A	D/C × C/C	25.115	19	0.163
8B	C/C × D/C	18.893	17	0.339
9A	D/C × C/C	42.234	39	0.674
9B	C/C × D/C	71.068	36	0.013
10A	D/C × C/C	26.987	39	0.162
10B	C/C × D/C	72.094	41	<0.010

of the twenty crosses, significant differences among bottle indices for females were found between duplicate bottles of the same cross.

(2) *Differences related to eclosion order.* In addition to the unexplained differences among bottles treated as similarly as possible, consistent differences in bristle index are also found within individual bottles. In each of the crosses raised by the bottle method, the first flies to eclose showed a higher mean bristle

index than the flies included in the second count, two days later. And these in turn had a higher mean index than flies included in the third count which was four days after the first. That is, in general, the bristle index decreased with eclosion order.

The underlying factor or factors which relate the bristle number of an individual fly to its eclosion order are unknown. They may include, among others, amount of moisture of food, presence of metabolic wastes of larvae, and amount of yeast available during all or part of the life of a larva. The yeast growth of a particular culture presumably varies partly with the number of larvae which have previously been feeding upon it, and perhaps partly with elapsed time, independently of larval population.

(3) *Difference related to area per eclosed larva.* It was found in all but one out of sixteen cases that bristle index was positively correlated with substrate area per eclosed larva. The positive correlation coefficients were found to vary from 0.479 in the male progeny of cross $2A(D/C♀ \times vg/vg♂)$ to 0.049 in the male progeny of cross $2B(vg/vg♀ \times D/C♂)$ (Table V). The excessive variability

TABLE V

Correlation coefficients between bristle index and substrate area

Cultured by	Cross	Female progeny	Male progeny
		r	*r*
Bottle method	1*A* (*D*/*C* × *vg*/*vg*)	0.303	0.160
Bottle method	1*B* (*vg*/*vg* × *D*/*C*)	0.471*	0.078*
Egg-count method	2*A* (*D*/*C* × *vg*/*vg*)	0.099*	0.479*
Egg-count method	2*B* (*vg*/*vg* × *D*/*C*)	0.085	0.049
Bottle method	4*A* (*D*/*C* × *M*-3/*C*)	0.092	0.084
Bottle method	4*B* (*M*-3/*C* × *D*/*C*)	0.309	0.321
Egg-count method	5*A* (*D*/*C* × *M*-3/*C*)	0.328	0.257
Egg-count method	5*B* (*M*-3/*C* × *D*/*C*)	−0.037	0.228

* Signifies cross with significantly different mean bristle index in reciprocal crosses.

of indices within crosses, and the influence on size of index of eclosion order, makes difficult an evaluation of the correlation between index and substrate area. A clarification should be based on data containing counts of daily hatches and not only, as in the data used, on complete hatches.

DISCUSSION

The original purpose of this investigation was to test for "maternal effect," *i.e.*, phenotypic effects expressed exclusively through or by the maternal parent, in the offspring of reciprocally crossed isogenic stocks. No significant differences were found between reciprocal crosses involving *M*-3, *M*-4, nor within Dichaete itself, but significant differences between reciprocal crosses involving *M*-2, *pyd*, and *vg* were found (as judged by mean posterior dorsocentral bristle index). However, two groups of data showed large and unexplained variation. These were the significant differences in mean bristle index of cultures *within* crosses, and the reversal in sign of the significant differences between crosses $1A(D/C♀ \times vg/vg♂)$ and $1B(vg/vg♀ \times D/C♂)$, and crosses $2A(D/C♀ \times vg/vg♂)$ and

$2B(vg/vg\,♀ \times D/C♂)$. In regard to the former, these significant differences arose among progeny of parental pairs of identical age and genotype. They arose in spite of efforts to produce, as nearly as possible, identical treatment of all flies. It must be concluded that these efforts were not sufficiently successful in making the environments of different sets of progeny homogeneous. Such factors as the condition of the substrate, the availability of yeast, and possibly the presence of bacteria and fungi in the fermenting medium were probably of influence on the bristle indices. With regard to the reversal in difference between bristle indices in the two sets of *vg*-Dichaete crosses, the unexplained variability just discussed may be the only cause of the opposing results. In addition, it should be pointed out that different culture methods were employed in the *vg*/Dichaete experiments. During larval life the closure of the experimental dishes in the "egg-count" method by means of cheesecloth and fingerbowls is considerably less tight than in the bottle method. It allows for more loss of substrate moisture and permits perhaps greater inoculation with bacteria and fungi. Furthermore, the greater area of substrate per larva in the "egg-count," as compared to the bottle method, presents a difference in environment, in degree if not in kind, to the developing larvae. The availability of yeast and the abundance of competitive organisms may be altered, as part of this environment, and in turn may influence the production of posterior dorsocentral bristles. Whether the effects of these two different environments is of such nature as to cause a significant reversal of mean bristle index between these two sets of reciprocal crosses is not conclusively shown in these data. The fact, however, that they may influence mean bristle index in experimental flies should not be ignored.

In the interest of more exact and uniform conditions, certain refinements of technique in future experiments may be mentioned here. It is well known that too few as well as too many larvae do not produce healthy culture conditions. Therefore, the optimal area per substrate per larva should be determined. In view of the fact that great variations in egg hatching are sometimes encountered, it is suggested that, where possible, counted larvae are chosen for experimental material rather than eggs. The substrate itself varies somewhat from culture to culture in the amount of moisture present and acidity (Bridges and Darby, 1933). Buffered substrate and controlled moisture conditions might aid in establishing more uniform culture conditions.

Differences between reciprocal crosses somewhat similar to those encountered in this work have also been described for *vg* by Hersh and Ward (1932), and Child (1939). No data were presented dealing with differences in wing size related to eclosion order, or to amount of substrate per larva, nor have tests of homogeneity been reported. The present work points to the necessity for extremely well controlled culture conditions and a searching statistical analysis, before conclusions concerning "maternal effect" can be accepted. In view of the residual, uncontrolled variability, the data obtained in this work are inconclusive as to the presence or absence of a "maternal effect."

Summary

1. To test for the existence of maternal effects in *Drosophila melanogaster,* approximately isogenic stocks of Dichaete, *pyd, vg, M*-3, *M*-4 were prepared.

Reciprocal crosses were made between each stock and Dichaete, and between Dichaete and non-Dichaete siblings. The results, as indicated by posterior dorsocentral "bristle indices" (*i.e.*, average number of dorsocentral bristles per hemithorax), of the Dichaete progeny show that no significant differences arose within six pairs of reciprocal crosses. The reciprocal crosses of four other pairs of experiments yielded significant differences in bristle indices. Two of these pairs were alike in type of crosses, but different in type of culture method. The sign of the differences in bristle indices was reversed in these two sets of experiments, the progeny with the higher indices coming from Dichaete mothers in one case, and from Dichaete fathers in the other.

2. Tests of homogeneity show that within crosses, all bottles of which have had identical treatment, differences in mean posterior dorsocentral bristle frequency occur which are greater than those to be expected on the basis of simple sampling errors.

3. The size of the bristle index of an individual fly depends partly upon its eclosion order, with those flies that eclose earliest having the highest bristle index.

4. The size of the bristle index depends partly on area of substrate available to the individual, although this dependence may be weak in many cases.

5. The lack of homogeneity in indices within crosses, and the reversal in direction of "maternal effect" in the pairs of crosses grown under two different culture conditions, suggest that external conditions during the development of the flies must be made more constant than heretofore, before conclusions as to the presence or absence of a "maternal effect" can be drawn.

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